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The occurrence of *Trypanosoma evansi* in buffaloes in Indonesia, estimated using various diagnostic tests

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SUMMARY

The prevalence and incidence of *Trypanosoma evansi* infections in village buffaloes in Central Java were estimated using parasitological tests, two antigen-detection ELISAs (2G6 Ag-ELISA and Tr7 Ag-ELISA), an antibody-detection ELISA (IgG ELISA) and a card agglutination test (CATT). Of 2387 village buffaloes tested in five districts, 4% (95% confidence interval [CI]: 3%, 5%) were positive with the microhaematocrit test (MHCT), 58% (95% CI: 56%, 60%) were positive with the 2G6 Ag-ELISA and 70% (95% CI: 68%, 72%) were positive with the Tr7 Ag-ELISA. An increasing prevalence with age was found and the proportion of positive buffaloes was highest in the over 84 months-old age-group (68%) with the 2G6 Ag-ELISA and in the 37–60 months-old age-group (78%) with the Tr7 Ag-ELISA. Parasitaemic buffaloes were found in more than half of the villages visited. Corrected village-specific prevalence values obtained with the two Ag-ELISAs ranged from 0% to over 100%, and prevalence differed significantly ($P \leqslant 0{\cdot}0001$) between villages in four of the five districts. Overall, 10% of buffaloes tested in markets were found to be parasitaemic and 39, 56 and 47% were found positive with the 2G6 Ag-ELISA, IgG ELISA and CATT, respectively. Incidence rates varied according to the test used and ranged from 0·22 (95% CI: 0·09, 0·44) to 0·44 (95% CI: 0·24, 0·76), per animal-year at risk, in two villages. The results highlight the importance of using validated diagnostic tests to obtain accurate estimates of prevalence and incidence. These parameters are needed, for example in mathematical models, for the development and evaluation of different control strategies for *T. evansi* infections in buffaloes.

INTRODUCTION

Trypanosoma evansi is a trypanosome which causes trypanosomosis ('surra') in livestock in many countries of Southeast Asia, Africa and South America [1]. Patterns of disease vary from acute epidemics with high case-fatality rates [2] to sub-clinical and chronic disease in endemic animal populations [3, 4]. In Indonesia, national losses associated with trypanosomosis in buffaloes and cattle have been estimated to be US $22·4 million per year [5], US $45/draught animal per year due to reduced work output [6] and US $9·14/beef animal [7]. Abortion [8], anoestrus [9] and weight loss [10] have also been associated with *T. evansi* infection. How-

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ever, total economic losses due to *T. evansi* in livestock throughout Southeast Asia, where large numbers of buffaloes and cattle are at risk of infection, are not known. Accurate prevalence and incidence values are needed to quantify these losses [3, 11], and to improve understanding of the occurrence of *T. evansi* in buffalo populations.

Several studies in Indonesia have used standard parasitological techniques (blood smears, the microhaematocrit test [MHCT] and the mouse inoculation test [MI]) to estimate the prevalence of *T. evansi* infections in buffalo and cattle and reported prevalence values in the range 0–5·8% and 1–5·7%, respectively [4, 12–14]. Parasitological tests are known to have a low diagnostic sensitivity, particularly for the detection of chronic trypanosomal infections which typically have low, fluctuating parasitaemias [15]; and higher prevalence values of *T. evansi* are found with antibody-detection tests. In one study, Payne and his colleagues [14] found 48% of the buffaloes tested were positive with an antibody-detection enzyme-linked immunosorbent assay (ELISA), but only 5·8% of the buffaloes were parasitologically positive. True prevalence values, therefore, are commonly underestimated in studies that use only parasitological tests. Moreover, surveys rarely use diagnostic tests that have been rigorously evaluated in terms of diagnostic sensitivity and specificity.

In addition to antibody-detection ELISAs, IgG-specific antibodies can also be detected using a card agglutination test (CATT) [16, 17]. However, serum antibodies are not detectable in early *T. evansi* infections, and antibody responses can persist after chemotherapy [18]. To improve the detection of current infections, antigen-detection ELISAs (Ag-ELISAs), which detect specific antigenic determinants of *T. evansi* and other trypanosomes in the circulation, have been developed [19–21]. Ag-ELISAs have been used to monitor tsetse-transmitted trypanosomosis control programmes in Uganda [22] and Zanzibar [23] but, to date, *T. evansi* Ag-ELISAs have not been used widely in Southeast Asia, where parasitological tests are still commonly used.

The main objective of this study was to estimate the prevalence and incidence of *T. evansi* infections in village buffaloes in Central Java, Indonesia, using antigen-detection and antibody-detection tests, in addition to standard parasitological tests. Incidence rates for *T. evansi* infections in buffaloes have not previously been reported and, in contrast to other studies, estimates of diagnostic sensitivity and specificity were used to calculate corrected estimates of true prevalence [24].

METHODS

Study areas and buffaloes sampled

The study area comprised five districts of Central Java, which has a high population of buffaloes, and five visits were conducted to each district. Prevalence was estimated by sampling buffaloes in different villages; incidence was estimated by re-sampling buffaloes in two villages. Buffaloes were also sampled at markets held weekly in each district to investigate the risk of *T. evansi* being introduced into villages through buffalo movements.

Cross-sectional study

Five visits were conducted at 3-monthly intervals (May 1994 to May 1995) to the districts of Batang (A), Pekalongan (B), Pemalang (C), Tegal (D) and Brebes (E). Villages were coded alphanumerically by district. During each visit, a target of approx. 100 buffaloes per district (the maximum number that could practically be tested) was sampled in villages that were chosen for convenience by government veterinary officers.

Longitudinal study

For estimation of incidence rate, 103 buffaloes were initially tested to identify 50 test-negative buffaloes (the maximum number that could feasibly be re-sampled during each visit). To have sufficient numbers of buffaloes, it was necessary to include buffaloes in two villages: Kebumen (Village A1, 50 buffaloes) in Batang district and Gejlig (Village B1, 53 buffaloes) in Pekalongan district. The buffaloes were over 1 year old, and identified either by ear-tattoo numbers or by numbers painted on the horn. Forty-nine buffaloes that had no detectable parasitaemia and that were negative with the 2G6 Ag-ELISA or Tr7 Ag-ELISA were selected; only 5 buffaloes were found to be negative with both Ag-ELISAs and 44 buffaloes were negative with one Ag-ELISA only.

Market study

Each market was visited once and the majority of buffaloes present were sampled (permission for sam-

pling was not given for a few buffaloes). Buffaloes were sampled in Batang market ($n = 30$) in July 1995, and in Pekalongan ($n = 65$), Pemalang ($n = 50$), Tegal ($n = 56$) and Brebes ($n = 38$) markets in September 1995.

Sample collection and diagnostic tests

Jugular blood was collected from each buffalo into plain Vacutainers and Vacutainers containing potassium ethylene diaminetetraacetic acid (EDTA) anticoagulant (Becton Dickinson), and the samples were transported on ice in a thermally-insulated box. Whole-blood samples were examined on the day of collection, and sera were kept on ice for 7–10 days until return to the laboratory, and then stored at -20 °C (sera handled by this method have been shown to give comparable results to samples frozen within 24 h of sampling [25]).

Standard MHCT and MI techniques [25] were used. All buffaloes were tested with the MHCT. One hundred white laboratory-bred mice were taken on each visit for MI tests. Each buffalo in the longitudinal study was tested by MI, and the remaining mice ($n \cong 50$) were used to test a random sample of buffaloes in the cross-sectional study. Every second market buffalo was tested by MI.

Sera were tested with the 2G6 Ag-ELISA, Tr7 Ag-ELISA and IgG ELISA, as described previously [26, 27], and with the CATT (Institute of Tropical Medicine, Laboratory of Serology, Nationalstraat 155, B-2000 Antwerp, Belgium). ELISA optical density values were expressed as a percentage of the positive control of each assay (i.e. percent positivity (PP) values) and a cut-off value of 20 PP was used [25]. With the CATT, a sample was positive if agglutination was observed, as recommended by the supplier.

All buffaloes in the longitudinal study were tested with both Ag-ELISAs, the IgG ELISA and CATT. In the cross-sectional study, all buffaloes ($n = 2387$) were tested with the 2G6 Ag-ELISA, but only buffaloes sampled in Visits 1–3 ($n = 1568$), and none of the market buffaloes were tested with the Tr7 Ag-ELISA because insufficient reagents were available.

Data analysis

Cross-sectional study

Corrected estimates of true prevalence were calculated from test prevalence values [24], using previously-derived point estimates of diagnostic sensitivity and specificity of the 2G6 Ag-ELISA (71%, 75%, respectively), Tr7 Ag-ELISA (81%, 78%, respectively), IgG ELISA (89%, 92%, respectively) and CATT (78%, 100%, respectively) [25]. Ninety-five percent confidence intervals (CIs) for prevalence values were calculated using the Confidence Interval Analysis software (CIA; © British Medical Journal, London) using either the exact binomial method or the Normal approximation method (when nP or $n[1-P] > 10$, where n = sample size, and P = estimated proportion) for simple random samples [28]. For villages in which the animal sampling fraction exceeded 10%, 95% CIs were calculated using an adjustment for large sampling fractions, as described by Thrusfield [24]. Estimates of prevalence were compared between villages and between markets using χ^2 tests for 2×2 and $2 \times k$ tables with an adjustment for continuity [29], and the linear association of age-specific prevalence values was assessed with the χ^2 test for trend [30] using the Epi Info software, version 6.03 [31]. Probability values (P) less than 0·05 were interpreted as statistically significant.

Longitudinal study

A buffalo was classified as newly infected if found to be parasitaemic or positive with the CATT or if there was a twofold increase from the initial Ag-ELISA or IgG ELISA PP value. The 20 PP cut-off value was not used in the ELISAs because several buffaloes had values above 20 PP with one of the Ag-ELISAs at the start of the study. Incidence rates were calculated per animal-year at risk, where the number of test-positive buffaloes was divided by the number of months at risk, and multiplied by 12 [24]. Numerators were assumed to be Poisson variables [32] and 95% confidence intervals, therefore, were taken from Poisson tables [33].

RESULTS

Details of the number of villages and buffaloes, and the number of villages visited and buffaloes sampled per district, are given in Table 1.

Cross-sectional study

Overall, 2387 village buffaloes were tested with the MHCT and 2G6 Ag-ELISA, 1568 buffaloes with the Tr7 Ag-ELISA and 360 buffaloes by MI. Buffaloes

Table 1. *Buffalo populations and number of villages in the five study districts of Central Java given with the numbers of villages with buffaloes, villages visited and buffaloes sampled*

District	Buffaloes*	Villages*	Villages with buffaloes*†	Villages visited	Buffaloes sampled
Batang	11523	242	186 (77)	10	387
Pekalongan	18369	274	192 (70)	12	422
Pemalang	25496	216	205 (95)	12	611
Tegal	20865	270	208 (77)	17	447
Brebes	16690	291	230 (79)	8	520
Total	92943	1293	1021 (79)	59	2387

* Data from [34].
† The percentage of villages with buffaloes per district is given in parentheses.

Table 2. *Corrected estimates of age-specific* Trypanosoma evansi *prevalence in buffaloes, obtained using two Ag-ELISAs*

		2G6 ELISA		Tr7 ELISA	
Age (months)	Number of buffaloes tested*	Uncorrected prevalence (%)	Corrected prevalence (%)	Uncorrected prevalence (%)	Corrected prevalence (%)
0–6	48 (31)	35	23	45	39
7–12	207 (146)	44	41	50	48
13–24	390 (307)	45	44	64	70
25–36	403 (330)	46	45	62	68
37–60	548 (434)	54	62	68	78
61–84	442 (364)	53	61	66	74
> 84	401 (268)	56	68	64	72
χ^2 value†		15·19	88·92	4·18	14·05
P value		0·0001	< 0·0001	0·041	0·0002

* Numbers of buffaloes tested with the Tr7 ELISA are given in parentheses.
† χ^2 test for trend, 6 D.F.

from 59 villages were sampled, representing 5·8 % of villages reported to keep buffaloes in the five study districts. Overall, the proportion of village buffaloes that were found to be positive, with 95 % confidence intervals given in brackets, was 4 % (3 %, 5 %) with the MHCT, 9 % (6 %, 12 %) by MI, 58 % (56 %, 60 %) with the 2G6 Ag-ELISA and 70 % (68 %, 72 %) with the Tr7 Ag-ELISA.

Age-specific prevalence

Test (i.e. uncorrected) prevalence and corrected estimates of true prevalence obtained with the two Ag-ELISAs for buffaloes of different ages are given in Table 2. A significant increase in prevalence with age was found with both Ag-ELISAs ($P < 0·05$). By 7–12 months of age, 41 % of buffaloes were found to be positive with the 2G6 Ag-ELISA, and 48 % were positive with the Tr7 Ag-ELISA. The highest proportion of positive buffaloes was found in the over 84 months-old age group (68 %) with the 2G6 Ag-ELISA and in the 37–60 months-old age-group (78 %) with the Tr7 Ag-ELISA.

Village-specific prevalence

Corrected estimates of true prevalence obtained with the two Ag-ELISAs ranged from 0 % to above 100 % and are shown in Table 3 (possible reasons for the values above 100 % are given in the discussion). Small numbers of buffaloes from some villages in District D were included because groups consisting of buffaloes rented from different village were sampled on a sugar plantation. At least one buffalo was found to be parasitaemic in more than half of the villages, and, in many villages higher prevalence values were obtained

Table 3. *Corrected estimates of* Trypanosoma evansi *prevalence in buffaloes of different villages,* obtained using two Ag-ELISAs*

Village code	Village buffaloes (*n*)	Buffaloes sampled (*n*)	2G6 Ag-ELISA§	Tr7 Ag-ELISA§
A1‡	98	30	104†	86 (76, 96)
A2‡	55	31	72 (62, 82)	88 (80, 96)
A3‡	63	27	74 (62, 86)	88 (79, 97)
A4‡	155	42	85 (76, 94)	83 (73, 93)
A5‡	155	44	59 (47, 71)	66 (54, 78)
A6‡	90	11	85 (65, 100)	86 (66, 100)
A7‡	200	48	46 (34, 58)	51 (39, 63)
A8‡	40	31	72 (64, 80)	61 (53, 69)
A9‡	119	56	39 (30, 48)	59 (50, 68)
B1‡	180	66	46 (36, 56)	97 (94, 100)
B2	115	46	54 (43, 65)	63 (52, 74)
B3‡	75	18	41 (21, 61)	76 (59, 93)
B4‡	70	33	46 (34, 58)	86 (77, 95)
B5‡	148	72	17 (11, 23)	19 (13, 25)
B6‡	80	26	73 (59, 87)	93 (85, 100)
B7	125	38	37 (24, 50)	75 (63, 87)
C1‡	325	36	41 (26, 56)	66 (51, 81)
C2‡	46	25	85 (76, 94)	51 (38, 64)
C3	172	50	67 (56, 78)	58 (46, 70)
C4‡	70	52	26 (20, 32)	61 (54, 68)
C5	450	78	63 (53, 73)	61 (51, 71)
C6‡	120	70	41 (34, 48)	63 (56, 70)
C7	36	23	50 (38, 62)	51 (39, 63)
C8	38	28	24 (16, 32)	66 (57, 75)
C9	78	4	54 (7, 93)	47 (7, 93)
D1‡	105	68	102†	100 (95, 100)
D2‡	135	11	24 (6, 61)	24 (6, 61)
D3	500	21	17 (5, 42)	68 (43, 85)
D4‡	28	24	83 (77, 89)	97 (94, 100)
D5	60	43	41 (33, 49)	69 (62, 76)
D6	170	70	46 (37, 53)	54 (45, 63)
D7	150	40	76 (65, 87)	95 (89, 100)
D8	4	4	54	47
D9	4	4	0	90
D10	21	4	0 (0, 60)	47 (7, 93)
D11	2	2	100	100
D12	35	2	54 (1, 99)	47 (1, 99)
D13	6	3	91 (59, 100)	76 (28, 100)
D14	50	5	33 (5, 85)	0 (0, 52)
E1	267	99	63 (55, 71)	75 (68, 82)
E2‡	280	173	74 (70, 78)	93 (91, 95)
E3‡	120	97	61 (57, 65)	85 (82, 88)

* Data shown for the 42 villages where buffaloes were tested with both Ag-ELISAs.
† Corrected values outside allowable range of 0–100%.
‡ Villages where at least one buffalo was found to be parasitaemic.
§ Prevalence (%) with 95% CI in parentheses.

with the Tr7 Ag-ELISA than with the 2G6 Ag-ELISA. There was significant inter-village variation in prevalence values obtained with the 2G6 Ag-ELISA in District A ($\chi^2 = 52{\cdot}30$, 8 D.F., $P < 0{\cdot}0001$), District B ($\chi^2 = 33{\cdot}73$, 6 D.F., $P < 0{\cdot}0001$), District C ($\chi^2 = 44{\cdot}35$, 7 D.F., $P < 0{\cdot}0001$), District D ($\chi^2 = 87{\cdot}48$, 6 D.F., $P < 0{\cdot}0001$) and District E ($\chi^2 = 6{\cdot}36$, 2 D.F., $P = 0{\cdot}042$). With the Tr7 Ag-

Table 4. *Estimates of* Trypanosoma evansi *prevalence in market buffaloes, obtained using various diagnostic tests**

Market district	Number of buffaloes sampled	Prevalence (%)			
		MHCT/MI‡	2G6 Ag-ELISA†	IgG ELISA†	CATT†
A	30	17	54	27	35
B	65	3	15	51	40
C	50	14	80	74	56
D	56	11	30	38	50
E	38	11	26	84	54
Total	239	10	39	56	47

* Buffaloes were not tested with the Tr7 Ag-ELISA.
† Corrected prevalence values.
‡ Uncorrected prevalence values; all buffaloes were tested with the MHCT and half the buffaloes were tested by MI.

Table 5. *Estimates of* Trypanosoma evansi *incidence rates, per animal-year at risk, in buffaloes in two villages, obtained using various diagnostic tests*

Village code	Incidence rate*				
	MHCT/MI	2G6 Ag-ELISA	Tr7 Ag-ELISA	IgG ELISA	CATT
A1	0·18 (0·04, 0·54)	0·19 (0·04, 0·56)	0·55 (0·24, 1·0)	0·19 (0·04, 0·56)	0·34 (0·11, 0·79)
B1	0·30 (0·10, 0·70)	0·25 (0·07, 0·64)	0·34 (0·11, 0·79)	0·24 (0·06, 0·60)	0·43 (0·17, 0·89)
A1 & B1	0·24 (0·11, 0·48)	0·22 (0·09, 0·44)	0·44 (0·24, 0·76)	0·22 (0·09, 0·44)	0·39 (0·19, 0·65)

* 95% CI given in parentheses.

ELISA, village prevalence values differed significantly within District A ($\chi^2 = 31{\cdot}36$, 8 D.F., $P = 0{\cdot}0001$), District B ($\chi^2 = 116{\cdot}48$, 6 D.F., $P < 0{\cdot}0001$), District D ($\chi^2 = 71{\cdot}27$, 6 D.F., $P < 0{\cdot}0001$) and District E ($\chi^2 = 17{\cdot}65$, 2 D.F., $P = 0{\cdot}0001$), but not within District C ($\chi^2 = 2{\cdot}51$, 7 D.F., $P = 0{\cdot}926$).

Market study

Of the 239 buffaloes sampled in the five markets, 114 were female and 125 were male, with ages ranging from 9 months to 13 years. Overall, 10% of these buffaloes were found to be parasitaemic, 39% were positive with the 2G6 Ag-ELISA, 56% were positive with the IgG ELISA, and 47% were positive with the CATT (Table 4). There were significant differences between prevalence values obtained in different markets with the 2G6 Ag-ELISA ($\chi^2 = 57{\cdot}53$, 4 D.F., $P < 0{\cdot}0001$) and with the IgG ELISA ($\chi^2 = 30{\cdot}51$, 4 D.F., $P < 0{\cdot}0001$), but not with the parasitological tests ($\chi^2 = 5{\cdot}73$, 4 D.F., $P = 0{\cdot}220$) or with the CATT ($\chi^2 = 5{\cdot}38$, 4 D.F., $P = 0{\cdot}251$). In Markets A and C, a higher prevalence was found with the 2G6 Ag-ELISA than with either of the two antibody-detection tests.

Longitudinal study

The corrected prevalence values obtained with the 2G6 Ag-ELISA and Tr7 Ag-ELISA in the two villages of the longitudinal study were 104% and 86% (Village A1) and 46% and 97% (Village B1), respectively. All buffaloes in the longitudinal study, except one, were female and their ages ranged from 18 months to 12 years old. By the last visit, 9 (18%) of the original 49 buffaloes had been sold. Of the 25 buffaloes that were found to be positive on at least one occasion, 16 buffaloes were positive with one test only: 3 buffaloes (MHCT/MI), 1 buffalo (2G6 Ag-ELISA), 4 buffaloes

(Tr7 Ag-ELISA), 1 buffalo (IgG ELISA) and 7 buffaloes (CATT). Higher sample incidence rates were obtained with the Tr7 Ag-ELISA and CATT than with the other tests (Table 5), however, the width of the associated 95% confidence intervals indicates that these estimates are imprecise (due to small sample sizes).

DISCUSSION

Trypanosoma evansi is reported to infect buffaloes and cattle throughout most of Indonesia [35]. Although the prevalence of *T. evansi* in buffaloes has been estimated in some areas [4, 12–14], inter-village variation has not been assessed, and tests with unknown diagnostic sensitivity and diagnostic specificity have been used. Buffaloes are kept in 70–95% of villages in the study area of Central Java, and buffaloes from 42 villages in 5 districts were tested with the MHCT, MI and two Ag-ELISAs. Overall, more buffaloes were found positive with the two Ag-ELISAs (58% and 70%) than with the parasitological tests (4% and 9%). Previous studies in Indonesia that used parasitological tests also found prevalence values of less than 10% [4, 13], and one study found 5·8% of buffaloes were MHCT-positive compared with 70% positive with an IgG ELISA [14].

No accurate 'gold standard' is currently available for the detection of *T. evansi* infections [18]. However, the diagnostic sensitivity and diagnostic specificity of the two Ag-ELISAs used in this study had been estimated previously [25], and therefore these parameter values were used to derive true prevalence values. An increasing prevalence with age was found with both Ag-ELISAs, but the peak proportion of positive buffaloes occurred earlier (37–60 months-old) with the Tr7 Ag-ELISA than with the 2G6 Ag-ELISA (over 84 months-old). The reason for this finding is not clear, but may be due to differences in the distribution of antigenaemia in infected buffaloes. The two Ag-ELISAs detect distinct antigenic determinants of *T. evansi* and antigenaemias have been shown to fluctuate during different stages of infection [25]. Although self-cure in experimentally-infected animals has been reported [36, 37], its occurrence in natural infections has not been demonstrated [1]. Therefore, these age-specific prevalence values are consistent with buffaloes remaining infected for many years (possibly with multiple re-infections) and surviving with chronic infections.

This is the first time that *T. evansi* Ag-ELISAs have been used in large-scale surveys in Southeast Asia. Previous studies used parasitological and/or antibody-detection tests and sampled livestock in a limited number of locations. At least one buffalo was found to be parasitaemic in more than half of the villages visited, indicating the potential for *T. evansi* transmission within these buffalo populations. The role of aparasitaemic, antigenaemic buffaloes in transmission is not known, but cattle experimentally infected with *T. brucei* and that did not have a detectable parasitaemia, were infective to tsetse flies [38]. Corrected village-specific prevalence values ranged from 0% to over 100% with the Ag-ELISAs, and prevalence varied significantly ($P \leqslant 0{\cdot}0001$) between villages in 4 of the 5 districts. Many surveys depend on convenience sampling because of financial and practical limitations, and in this study villages with higher numbers of buffaloes and with co-operative farmers were more likely to be visited than other villages. The results suggest that the precision of results is affected by intra-village and inter-village variation in the proportions of buffaloes that are test-positive, and therefore that both sources of variation should be considered in future surveys [39].

There are several possible reasons for the observed inter-village variation in *T. evansi* prevalence. Increasing prevalence with age was found in this study and has been reported previously [4, 14], and age is recognized as a confounder for trypanosomosis serology [40]. In most villages, buffaloes were adult females used for draught power, although in a few villages younger buffaloes were kept for fattening. Many factors, for example group size and potential contact with fly populations, may influence the transmission dynamics of *T. evansi*. In the study area, farmers typically owned 1–5 buffaloes, as in other areas of Indonesia [41], and while some buffaloes were kept in small traditional stalls, most were kept in groups of up to 100 which were grazed together and kept in communal animal houses (*kandangs*). Furthermore, for 1–6 months of the year, buffaloes were worked in rice fields where there is greater potential exposure to biting flies; some buffaloes were sampled during this period. Tabanids are important vectors of *T. evansi* [1], and infection rates of Tabanid populations vary between villages [42]. Moreover, transmission efficiency differs between Tabanid species and other biting flies [43]. However, in Indonesia limited data are available on current fly populations and *T. evansi* infections within these populations.

Differences were observed between the results obtained with the various diagnostic tests. In general, higher village-specific prevalence values were found with the Tr7 Ag-ELISA than with the 2G6 Ag-ELISA. True prevalence was underestimated by test prevalence at higher prevalence values, and over-estimated at lower prevalence values. Correction of test prevalence values should, theoretically, eliminate differences between test prevalence values, if valid and precise estimates of the diagnostic sensitivity and diagnostic specificity of each test are used. The diagnostic sensitivity of these tests had been estimated previously using parasitaemic buffaloes living in Indonesia, and the diagnostic specificity had been estimated using buffaloes living in Australia where *T. evansi* is not reported to occur [25]. If these populations were not fully representative of the village buffalo groups tested, then differences between the values obtained with these two tests would reflect an underlying imprecision of their test parameter values which would explain why some corrected values were outside the allowable range of 0–100%.

A rapid turnover of buffaloes has been reported to occur in West Java [44] where buffaloes are kept principally for fattening and as a financial investment. By contrast, buffaloes tend to be kept longer in Central Java where they are used primarily for draught power. During the study, farmers reported that sick buffaloes are sold quickly and at a reduced price to minimize the risk of substantial financial loss if a buffalo dies, a practice which also occurs in West Java [44]. Buffalo movements are likely to be important in the spread of different strains of *T. evansi* [6] and 10% of the buffaloes in the markets visited were found to be parasitaemic and 39% were antigenaemic. An outbreak of trypanosomosis on Madura Island was attributed to the movement from Java of *T. evansi*-infected horses which were sold through markets [45]. The majority of market buffaloes tested were older buffaloes, which are more likely to have *T. evansi* infections, and there was significant inter-market variation in prevalence. In two markets, more buffaloes were antigenaemic than antibody-positive which is indicative of recent infections.

Incidence rates have not previously been reported for *T. evansi* infections in buffaloes, and rarely for other trypanosomal infections. Overall, incidence rates ranged from 0·22 (0·09, 0·44) to 0·44 (0·24, 0·76), per animal-year at risk, and higher rates were obtained with the Tr7 Ag-ELISA and CATT than with the parasitological tests, 2G6 Ag-ELISA or IgG ELISA. However, the estimates were imprecise due to small sample sizes and, unlike prevalence values, these rates were not corrected to account for the diagnostic sensitivity and specificity of the tests. Furthermore, it was difficult to identify buffaloes that were not antigenaemic because of the high prevalence of *T. evansi* in the study areas. Within these limitations, the results suggest that, if infection is independent of age and persists then buffaloes in these villages were likely to become infected, on average, within approximately 4 years (i.e. equivalent to 0·25 per animal-year at risk, assuming constant risk). A study of 45 Australian buffaloes that were imported into Java found that 31% of the buffaloes were parasitaemic, and 25% were positive with an IgG ELISA by 13 weeks after importation [14].

This study estimated the prevalence and incidence of *T. evansi* infections in village buffaloes in five districts of Central Java. Further studies are needed to provide additional data for other areas of Indonesia and elsewhere in Southeast Asia which, together with the values obtained in this study, could be used in mathematical models being developed for the design of cost-effective control strategies.

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REFERENCES

1. Luckins AG. *Trypanosoma evansi* in Asia. Parasitol Today 1988; **4**: 137–41.
2. Lun Z-R, Fang Y, Wang C-J, Brun R. Trypanosomiasis of domestic animals in China. Parasitol Today 1993; **9**: 41–5.
3. Wilson AJ. some observations on the epidemiology of the animal trypanosomiases with particular reference to *T. evansi*. In: Campbell RSF, ed. A course manual in veterinary epidemiology. Canberra: Australian University International Development Programme, 1983: 201–10.
4. Partoutomo S, Soleh M, Politedy F, et al. The epidemiology of *Trypanosoma evansi* and *Trypanosoma*

theileri in cattle and buffalo in smallholder farms in Java. Penyakit Hewan 1994; **26**: 41–6.
5. Ronohardjo P, Wilson AJ, Partoutomo S, Hirst RG. Some aspects of the epidemiology and economics of important diseases of large ruminants in Indonesia. In: Proceedings of the Fourth International Symposium on Veterinary Epidemiology and Economics, Singapore, 1986: 303–5.
6. Rukmana MP. Microhaematocrit method as a new technology in diagnosing surra and its relevancy to livestock economics [PhD thesis]. Bandung, Indonesia: Universitas Padjadjaran, 1979.
7. Payne RC, Sukanto IP, Partoutomo S, Sitepu P, Jones TW. Effect of suramin treatment on the productivity of feedlot cattle in a *Trypanosoma evansi* endemic area of Indonesia. Trop Anim Health Prod 1994; **26**: 35–6.
8. Lohr KF, Pholpark S, Siriwan P, Leesirikul N, Srikitjakarn L, Staak C. *Trypanosoma evansi* infection in buffaloes in north-east Thailand. II Abortions. Trop Anim Health Prod 1986; **18**: 103–8.
9. Payne RC, Sukanto IP, Bazeley K, Jones TW. The effect of *Trypanosoma evansi* infection on the oestrous cycle of Friesian Holstein heifers. Vet Parasitol 1993; **51**: 1–11.
10. Payne RC, Sukanto IP, Partoutomo S, Polytedi F. Experimental infection of Friesian Holstein calves with an Indonesian isolate of *Trypanosoma evansi*. Trop Med Parasitol 1992; **43**: .
11. Hutabarat TSPN, Holden SJ. Inclusion of economic information in national animal health planning in Indonesia. In: Martin SW, ed. Proceedings of the Sixth International Symposium on Veterinary Epidemiology and Economics, Ottawa, 1991: 201–3.
12. Adiwinata RT, Dachlan A. A brief note on surra in Indonesia. ELVEKA Folia Vet 1969; **3**: 10–5.
13. Siswansyah DD, Tarmudji P. Prevalensi trypanosomiasis pada pemeriksaan darah sapi dan kerbau di Kalimantan Selatan dengan metode mikrohematokrit. Penyakit Hewan 1989; **21**: 118–22.
14. Payne RC, Sukanto IP, Djauhari D, et al. *Trypanosoma evansi* infection in cattle, buffaloes and horses in Indonesia. Vet Parasitol 1991; **38**: 109–19.
15. Nantulya VM. Trypanosomiasis in domestic animals: the problem of diagnosis. Rev Sci Tech OIE (Off Int Epizoot) 1990; **9**: 357–67.
16. Bajyana-Songa E, Hamers-Casterman C, Hamers R, et al. The use of the card agglutination test (Testryp CATT) for the detection of *Trypanosoma evansi* infection: a comparison with other trypanosomiasis diagnostic tests under field conditions in Thailand. Ann Soc Belge Méd Trop 1987; **67**: 137–48.
17. Wuyts N, Chokesajjawatee N, Sarataphan N, Panyim S. PCR amplification of crude blood on microscope slides in the diagnosis of *Trypanosoma evansi* infection in diary cattle. Ann Soc Belge Méd Trop 1995; **75**: 229–37.
18. Luckins AG. Diagnostic methods for trypanosomiasis in livestock. World Anim Rev 1992; **71**: 15–20.
19. Rae PF, Thrusfield MV, Higgins A, Aitken CGG, Jones TW, Luckins AG. Evaluation of enzyme immunoassays in the diagnosis of camel (*Camelus dromedarius*) trypanosomiasis: a preliminary investigation. Epidemiol Infect 1989; **102**: 297–307.
20. Nantulya VM, Lindqvist KJ. Antigen detection enzyme immunoassays for the diagnosis of *Trypanosoma vivax*, *T. congolense* and *T. brucei* infections in cattle. Trop Med Parasitol 1989; **40**: 267–72.
21. Nantulya VM, Bajyana Songa E, Hamers R. Detection of circulating trypanosomal antigens in *Tryypanosoma evansi*-infected animals using a *T. brucei* group-specific monoclonal antibody. Trop Med Parasitol 1989; **40**: 263–6.
22. Okuna NM, Mayende JSP, Maagona J. The validation of an Ag-ELISA for the diagnosis of trypanosomiasis in cattle in Uganda and its use in assessing the efficacy of a control programme. In: Improving the diagnosis and control of trypanosomiasis and other vector-borne diseases of African livestock using immunoassay methods. IAEA-TECDOC-707. Vienna: International Atomic Energy Agency, 1993: 69–77.
23. Mbwambo HA, Mpokwa MH. Antigen-detection enzyme-linked immunoassay (Ag-ELISA) as an aid to the diagnosis of animal trypanosomiasis in Tanzania. In: Improving the diagnosis and control of trypanosomiasis and other vector-borne diseases of African livestock using immunoassay methods. IAEA-TECDOC-707. Vienna: International Atomic Energy Agency, 1993: 79–86.
24. Thrusfield M. Veterinary epidemiology, revised 2nd ed. Oxford: Blackwell Science, 1997: 266–84.
25. Davison HC, Evaluation of diagnostic tests for *Trypanosoma evansi* and their application in epidemiological studies in Indonesia [PhD thesis]. Edinburgh: University of Edinburgh, 1997.
26. Davison HC, Husein A, Muharsini S, Thrusfield MV, Rae PF, Partoutomo S, Luckins AG. Evaluation of *Trypanosoma evansi* antigen-ELISAs. II Field studies – (b) data analysis. In: Husein A, Davison HC, Luckins AG, Partoutomo S, Thrusfield MV, eds. Proceedings of a seminar on diagnostic techniques for *Trypanosoma evansi* in Indonesia. Bogor, Indonesia: Research Institute for Veterinary Science, 1996: 34–40.
27. Davison HC, Muharsini S, Thrusfield MV, Partoutomo S, Rae PF, Luckins AG. Evaluation of *Trypanosoma evansi* antigen-ELISAs. I Experimental studies. In: Husein A, Davison HC, Luckins AG, Partoutomo S, Thrusfield MV, eds. Proceedings of a seminar on diagnostic techniques for *Trypanosoma evansi* in Indonesia. Bogor, Indonesia: Research Institute for Veterinary Science, 1996: 23–8.
28. Gardner MJ, Altman DG. Statistics with confidence – confidence intervals and statistical guidelines. London: British Medical Journal, 1989: 28–33.
29. Bailey NTJ. Statistical methods in biology, 2nd edn. London: Hodder and Stoughton, 1981: 58.
30. Mantel N. Chi-square tests with one degree of freedom: extension of the Mantel–Haenszel procedure. J Am Stat Assoc 1963; **58**: 690–700.
31. Sullivan K, Fagan RF, Arner TG Epi Info Version 6.03 A word processing, database and statistics programme

for epidemiology on microcomputers. Centers for Disease Control and Prevention, Atlanta, Georgia, USA, 1995.
32. Kahn HA, Sempos CT. Statistical methods in epidemiology. Oxford: Oxford University Press, 1989 (Monographs in Epidemiology and Biostatistics; vol 12).
33. Diem K. Documenta Geigy scientific tables, 6th edn. Manchester: Geigy Pharmaceutical Company Ltd, 1962: 107.
34. Buku Statistik Kesehatan Hewan. Jakarta: Direktorat Jenderal Peternakan, Government of Indonesia, 1989.
35. Anonymous. Bulletin epidemiologi veteriner. Jakarta: Direktorat Bina Kesehatan Hewan, Direktorat Jenderal Peternakan, Government of Indonesia, 1993: 16–28.
36. Williams DJL, Naessens J, Scott JR, McOdimba FA. Analysis of peripheral leucocyte populations in N'Dama and Boran cattle following a rechallenge infection with *Trypanosoma congolense*. Parasite Immunol 1991; **13**: 171–85.
37. Onah D. *Trypanosoma evansi*: Immune responses and immunosuppression during experimental infection in sheep [PhD thesis]. Edinburgh, University of Edinburgh, 1992.
38. Masake RA, Moloo SK, Nantulya VM, Minja SH, Makau JM, Njuguna JT. Comparative sensitivity of antigen-detection enzyme immunosorbent assay and the microhaematocrit centrifugation technique in the diagnosis of *Trypanosoma brucei* infections in cattle. Vet Parasitol 1995; **56**: 37–46.
39. Smith RD. Veterinary clinical epidemiology – a problem oriented approach, 2nd edn. Boca Raton, Florida: CRC Press, 1995.
40. Greiner M, Shivarama Bhat T, Patzelt RJ, et al. Impact of biological factors on the interpretation of bovine trypanosomosis serology. Prev Vet Med 1997; **30**: 61–73.
41. Anonymous. A review of the livestock sector in the Republic of Indonesia, vols 1, 2. USA: Winrock International Institute for Agricultural Development, 1986.
42. Hartini S, Aziz J. *Trypanosoma evansi* in *Tabanus rubidus* in Jonggol, West Java. Malayan Vet J 1991; **3**: 31–2.
43. Foil LD. Tabanids as vectors of disease agents. Parasitol Today 1989; **5**: 88–96.
44. Pearce MC. Consultant's Report (M2-94-01) for MCF Project Extension of ODA Animal Health Project April 1993–March 1994. Bogor: Indonesia International Animal Science Research and Development Foundation, 1994: 1–41.
45. Payne RC, Sukanto IP, Graydon R, Saroso H, Jusuf SH. An outbreak of trypanosomiasis caused by *Trypanosoma evansi* on the island of Madura, Indonesia. Trop Med Parasitol 1990; **41**: 445–6.